In the Specification:

Please insert the following paragraph onto the first page of the specification immediately below the title and above the "Field of the Invention."

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of United States Patent No. 6,238,687, filed on April 5, 1999, which is a continuation-in-part of Application Serial No. PCT/US98/07585, filed on April 14, 1998, which is a continuation-in-part of United States Application Patent No. 5,912,225, filed on April 14, 1997, and Applicants claim benefit thereof and priority thereto.

Please replace paragraphs 4, 57, 67, 79, 109, 194, 215, 232, 234, 239, 268 and 269 with the amended versions shown as Replacement Paragraphs to the Specification, marked up to show changes made relative to the immediate prior version. No new matter is presented in the replacement paragraphs.

[0004] To circumvent these problems, synthetic nerve guide conduits (NGCs) have been developed to bridge the nerve gaps by inserting the severed nerve stumps into the two ends of the conduit. The purpose of such nerve guide conduits is to encourage the processes of neuronal growth and regeneration of nerve function. To be effective, these NGCs must be made from materials that meet a wide range of biological and physicochemical prerequisites. The material must be nontoxic, non-carcinogenic, non-antigenic, and must demonstrate favorable mechanical properties such as flexibility, suturability, and amenability to custom fabrication. One example of NGC materials that have been used in combination with sutures is ~~siliconos~~ silicone rubber as taught by R. D. Midgley and F. M. Woolhouse, Silicone Rubber Sheathing as an Adjunct to Neural Anastomosis, Surgical Clinic of North America 48, 1149 (1968). Silicone has the

disadvantages of being impermeable and ~~non-adsorbable~~ non-degradable. The use of biodegradable polymers to fabricate NCGCs has been the focus for many years. The use of bioresorbable, polyglactin mesh tubing was reported by Molander et al. in Vol. 5, Muscle & Nerve. pp. 54-58 (1982). The use of porous acrylic copolymer tubes in nerve regeneration was disclosed by Uzman et al. in Vol. 9, Journal of Neuroscience Research. pp. 325-338 (1983). Bioresorbable nerve guidance channels of polyesters and other polymers have been reported by Nyilas et al. in Vol. 29, Transactions Am. Soc. Artif. Internal Organs. pp. 307-313 (1983) and in U.S. Pat. No. 4,534,349 issued to Barrows in 1985. After accomplishing their function, these guides are supposed to gradually disappear from the host. These biodegradable tubes have the advantage over using biodurable NGCs in dispensing with a second surgery to remove implanted tubes. Such a second operation is often necessary after biodurable NGCs are implanted in order to eliminate the potential problems, such as chronic tissue response and nerve compression, caused by the long-term presence of a rigid tube in the body. In contrast, after a biodegradable tube has accomplished its function, the guide gradually disappears from the host.

[0057] Conveniently, the cationic polymer ~~of lipid~~ or lipid comprises ~~Transfast or GenePORTER~~ TRANSFAST or GENEPORTER.

[0067] Advantageously, at least ~~100 µm~~ 100 µg of protein is loaded per 10 mm of conduit.

[0079] Preferably, the method comprises the step of, after adding the first and second non-solvents, increasing the concentration of the second ~~solvent~~ non-solvent.

[0109] **FIG. 3** shows schematic ternary phase diagrams representing the phase changes that occur during the process of immersion precipitation of: (a)P(BHET-EOP)/$CHCl_3$/MeOH system;

and (b) P(BHET-EOP)/$CHCl_3$/H_2O system. The cloud point data is assumed to represent the ~~bimodal~~ binodal demixing boundary, considering the relatively long time scale required for the demixing process to occur in this system. The gelation point for the polymer-$CHCl_3$ system was estimated at 69%(w/w), while the gelation boundary is schematic. The two phase diagrams represent the two extreme cases of immersion precipitation in pure methanol or pure water baths respectively. In the case of water-methanol mixtures, the demixing boundary is expected to shift to intermediate coordinates. This results in changes in the length of time required for the phase separation process to occur, if at all.

[0194] The porosity of the polymer surfaces obtained via the various procedures was estimated by generating a random array of 20 coordinates using a microprocessor, upon which the respective SEM micrographs (at 1500× magnification) were superimposed. The surface porosity, expressed as percentage of surface area covered by pores was calculated by employing the statistically derived formula:

~~surface porosity (%) number of coordinates within pore region/total number of coordinates×100%~~ surface porosity (%) = (number of coordinates within pore region/total number of coordinates)×100%

[0215] To measure weight loss, conduits were placed in phosphate buffer pH 7.4 at 37° C. At selected time points, the solution was removed and the conduits were dried to constant weight under vacuum and ~~weighted~~ weighed. The percentage of weight loss (WL%) was calculated according to the following equation:

$$WL(\%)=100\times(Wo-Wr)/Wo$$

[0232] Transverse sections through the mid-point of the 10 mm gaps were analyzed to determine the number of myelinated axons one and three months after implantation (Table 1). The Type I conduits with a higher surface porosity had a higher fibre population than Type II conduit, which is very close to the value from the normal nerves by 3 months pos-implantation.

TABLE 1

Morphemetric Analysis of the Regenerated Nerves at the Midpoint of Conduits With Different Surface Porosity.

PPE NGC	Porosity	Implantation Period	N	Fibre Population
Type I	35%	1 month	4	3758 ± 1043
Type II	8%	1 month	7	2928 ± 1293
Type I	35%	3 months	4	8080 ± 240
Type II	8%	3 months	11	6684 ± 2155
Normal Nerve	—	—	7	7991 ± 257

~~Example VIII~~ Example VII

Nerve Regeneration within Conduits Loaded with Polyethylenimine/DNA Complexes

[0234] Plasmid DNA was diluted in 5% glucose to the chosen concentrations. PEI ([[25 k]] 25 kDa, Sigma) was used as 0.1 M aqueous stock solution. Relative amounts of PEI to DNA were 10 equivalents of PEI nitrogen per DNA phosphate (N/P=10/1). Complexes were formed by addition the appropriate amount of PEI solution into DNA solution, mixing with brief vortex and waiting for 15 min at room temperature. For each loading, 20 µl of 5% glucose solution containing 4 µg of plasmid DNA complexed with PEI or other DNA carriers were used per conduit.

[0238] In addition to polyethylenimine, other cationic polymers or lipids may also be used to deliver genes through endocytosis and retrograde axonal transport. The gene expression of human Bcl-2 was used to compare their relative transfer efficiency. Optimal ratios between DNA and poly-L-lysine, chitosan, TRANSFAST or GENEPORTER were tested in agarose gel electrophoresis and COS7 cell transfection. The same amounts of pcDNA3/Bcl-2 were then complexed with those polymers and lipids under the conditions optimized for each individual agent and injected into the tongue. The proteins extracted from the brainstem 2 days after injection were subjected to western blotting (**FIG. 18**). The highest expression level was obtained with PEI, which is at least two times higher than that mediated with poly-L-lysine and chitosan as showed by densitomeric analysis. Very weak expression was observed with two cationic lipids, TRANSFAST and GENEPORTER. Injection of free plasmid DNA into the tongue did not gain detectable Bcl-2 expression in the brainstem.

~~Example VII~~ Example VIII

Nerve Regeneration within Conduits Loaded with NGF Microspheres

[0268] 20. Wan, [[AAC]] ACA, Mao H-Q, Wang S, Leong KW, Ong LKLL, Hanry Y.

[0269] Fabrication of poly(phosphoester) nerve guides by immersion precipitation and the control of porosity, Biomaterials, ~~2000, in press~~ 2001; 22: 1147-1156.